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(57) Abstract

Human antibody preparations enriched for antibodies that bind to human T-cell receptor peptide sequential determinants are disclosed. Also disclosed are methods for making such antibody preparations from human plasma by immunoaffinity purification using either recombinant Tcr protein or Tcr variable region peptides. Purification of Tcr antibodies from human or animal antibody mixtures is accomplished by immunoaffinity procedures using recombinant human T-cell receptor proteins. The affinity purified antibodies can bind to intact membrane-bound Tcr using fluorescence activated cell sorting (FACS) and can inhibit T cell proliferation following stimulation by mitogen. Affinity purified human polyclonal antibodies can inhibit lytic activity of natural killer cells on a monoclonal T-cell line. The antibody preparations can be used for diagnosis, monitoring and therapy of various autoimmune-related syndromes.

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HUMAN ANTIBODIES TO T-CELL RECEPTOR PEPTIDES AND METHODS FOR THEIR PREPARATION

This application is a continuation-in-part of U.S. Serial Number 08/341,157, filed November 16, 1994.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to antibody preparations that are enriched for antibodies capable of binding to certain T-lymphocytes. More particularly, the invention relates to human antibody preparations that are enriched for antibodies which bind to human T-cell receptor variable region peptides and conformational determinants. The invention further relates to certain methods for making such antibody preparations. These antibody preparations are useful for diagnosing immune system disorders, such as autoimmune diseases and graft versus host disease ("GVHD"). They also have potential therapeutic value in treating these diseases.

2. Description of the Background Art

T-lymphocytes (also referred to herein as T-cells) are involved in cell-mediated immunity. T-cells have been implicated in various immune system disorders, such as autoimmune related syndromes, including classic autoimmune diseases and GVHD. In recent years, it has

been observed that high dosage administration of intravenous immunoglobulins ("IVIG") has profound effects on a wide variety of immune system-related diseases. These diseases include non-hematologic autoimmune diseases as well as immunohematologic and other diseases with immunopathologic features. Patients suffering from both chronic autoimmune disease and acute GVHD have been found to respond to IVIG treatment regimens. Clinical improvements have been attributed (at least in part) to certain antibodies contained in these preparations.

T-cells have on their surface a T-cell receptor ("Tcr") which is responsible for the immunological specificity of the cells. The Tcr is associated with polypeptides which form the CD3 complex. The Tcr recognizes processed antigen associated with a molecule which is a product of the major histocompatibility complex ("MHC"). The polypeptide chains for the antigen-binding portion of the Tcr are encoded by four different gene loci, designated α, β, δ and γ. A given T-cell will express either an α/β or a δ/γ receptor. The Tcr's of the great majority of peripheral T-cells are composed of polypeptide products of the α/β gene loci.

The genes encoding the Tcr are similar to those which encode antibody. They consist of multiple V, D and J segments which become recombined during T-cell development to produce functional VDJ or VJ genes. The genes encode the N-terminal variable (V) domains of the Tcr. The human genome contains approximately 100 Vα genes and between 50 and 100 Vβ genes.

Kawasaki's disease and multiple sclerosis ("MS") are two examples of autoimmune diseases in which T-

cells are clearly implicated. For example, patients with acute Kawasaki's disease demonstrate significantly elevated levels of circulating T-cells bearing the products Vβ2 and Vβ8.1 genes, indicative of specific up-regulation. The use of IVIG results in significant clinical improvement and a return to near-normal levels of T-cell subsets. Serum from patients suffering from MS have been found to possess elevated levels of T-cells reactive with myelin base proteins, and these T-cells tend to express products of the Tcr Vβ 5.2 and 6.1 genes rather than the entire pool of Tcr Vβ genes.

Human Tcr peptides have been used as immunogens to produce animal antisera. Schluter, S.F. and Marchalonis, J.J., Proc. Natl. Acad. Sci. USA, 83:1872-76 (1986). A need exists for human antibodies to human Tcr peptide sequences for use in diagnosing certain immune system disorders and for therapeutic use in the treatment of such disorders.

SUMMARY OF THE INVENTION

The present invention provides a human antibody preparation which is enriched for antibodies that bind to T cell receptor variable region peptides and conformational determinants. Also disclosed is a method of using such antibody preparation for the diagnosis or treatment of an autoimmune disease or condition or GVHD which is associated with an elevation of T-lymphocytes bearing a particular Tcr peptide or conformational determinant.

The invention further provides methods for making antibody preparations enriched for antibodies with binding specificities to these Tcr autoantigenic variable region epitopes. In one embodiment, the method involves combining, under antibody-antigen

binding conditions, (i) a Cohn human plasma fraction which contains antibodies that bind to a human Tcr peptide sequence and (ii) a solid support to which Tcr peptide has been immobilized; separating unbound proteins from the solid support; and eluting bound antibodies from the solid support under conditions which break the antibody-antigen bonds, thereby forming an antibody preparation which is enriched for antibodies that bind to a human Tcr peptide sequence. In another embodiment, the method involves combining, under antibody-antigen binding conditions (i) a human or animal antibody mixture which contains antibodies that bind to a human Tcr peptide or conformational determinant and (ii) a solid support to which a recombinant human Tcr protein has been immobilized; separating unbound proteins from the solid support; and eluting bound antibodies from the solid support under conditions which break the antibody-antigen bonds, thereby forming an antibody preparation which is enriched for antibodies that bind to a human Tcr peptide or conformational determinant.

The novel antibody preparations of this invention have utility in diagnosing or monitoring the progress of human immune system disorders, such as autoimmune diseases and GVHD. These antibody preparations further have value as human therapeutic agents. As a result of the work reported herein, it is believed that IVIG preparations are effective in treating immune disorders because they contain autoantibodies which bind to Tcr protein on the surfaces of T-lymphocytes. The antibody preparations of the present invention provide more potent and selective therapeutic agents.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a fluorescent activated cell sorter (FACS) scan of Jurkat cells treated with antibody free reagents (negative control).

Figure 2 is a FACS scan of Jurkat cells treated with rabbit anti-scTcr antiserum (positive control).

Figure 3 is a FACS scan of Jurkat cells treated with unpurified IVIG.

Figure 4 is a FACS scan of Jurkat cells treated with IVIG purified by immunoaffinity chromatography using immobilized scTcr.

Figure 5 represents ELISA results showing the reactivity of Cohn Fraction I + III with scTcr and various Tcr peptides.

Figure 6 represents ELISA results showing the reactivities of sera from patients with rheumatoid arthritis with scTcr.

Figure 7 represents ELISA results showing the reactivities of sera from patients with SLE with scTcr.

Figure 8 is a graph illustrating the results of cell proliferation assays comparing the inhibitory effects (shown as percent inhibition) of increasing concentrations of purified anti-β3 antibodies and a commercial intravenous immunoglobulin (IVIG).

Figure 9 is an overview depiction of the experimental design of an experiment to study the effects of purified antibodies in modulating T cell activity via interaction with the TcR.

Figure 10 is a graph illustrating the inhibition of lymphocyte killing of tumor targets by anti-TcR antibodies purified from a commercial intravenous immunoglobulin (IVIG).

DETAILED DESCRIPTION

The novel antibody preparations of this invention are advantageously prepared from human antibody mixtures which contain antibodies having the desired reactivities. Healthy humans, as well as those suffering from autoimmune diseases and conditions, such as systemic lupus erythematosis and rheumatoid arthritis, and autoimmune related syndromes, such as GVHD, contain autoantibodies directed against peptide and conformational determinants occurring in Tcr proteins. See, Marchalonis et al., Proc. Natl. Acad. Sci., 89:3325-3329 (1992) and Marchalonis et al., Gerontology, 39:65-79 (1993). Thus, pooled serum from healthy individuals or those suffering from autoimmune diseases may be used as sources for the antibody preparations of this invention. Commercially available plasma products or fractions also advantageously may be used as the source for these antibody preparations. For example, immune serum globulin products and commercial IVIG preparations such as Gammagard®, available from Baxter Healthcare International, and Sandoglobulin, available from Sandoz Pharmaceuticals, can be used as a starting material. Various Cohn plasma fractions (Cohn et al., J. Am. Chem. Soc., 68:459 (1946)) have been found to contain autoantibodies directed to Tcr peptide sequences. Cohn Fractions I and III, which are currently discarded, as well as Cohn Fraction II, can be used as starting materials for the production of the antibody preparations of this invention.

The novel antibody preparations are conveniently obtained by immunoaffinity purification using immobilized Tcr peptides or recombinant human Tcr proteins. The latter may be preferred because of their

ability to bind to antibodies through both peptide and conformational determinants. Tcr peptides are immobilized by bonding them to a conventional solid support, such as agarose beads, using well known conventional methods. See, Marchalonis et al., 1992, *supra*. Various peptides corresponding to sequences of the Tcr antigen binding region can be used in the immunoaffinity purification procedure. These peptides are preferably encoded by Vα and/or Vβ genes, but may also be encoded by Vδ and/or Vγ genes. Tcr peptides chosen represent regions of the Tcr found to be autoimmunogenic as manifested by production of IgG and IgM autoantibodies. Marchalonis et al., 1992, supra. Recombinant Tcr proteins contain these sequences. Lake et al., Biochem. Biophys. Res. Comm., 201 (301): 1502-109 (1994).

The Tcr peptides used for the antibody purifications may be chemically synthesized using conventional peptide synthesis techniques. Recombinant human Tcr proteins may be made by various recombinant DNA procedures, e.g., by cDNA cloning techniques using messenger RNA obtained from commercially-available T-cell lines, or from T-cells cultured from normal blood or blood from patients suffering from autoimmune diseases. Genes from the Vα, Vβ, Vδ and Vγ loci have been sequenced (Toyonaga, B. And Mak, T.W., Ann. Rev. Immunol., 5, 585-620 (1987)), and these sequences may be utilized for designing PCR primers and probes for amplifying and identifying cDNA clones.

As indicated above, particular autoimmune diseases have been associated with elevated levels of circulating T-cells bearing the products of specific Tcr genes. As used herein, a Tcr protein, peptide or conformational determinant is said to be associated

with a particular immune system disorder or condition when T-cells bearing that determinant are elevated in patients having the disorder or condition. Thus, T-cells bearing the products of the Vβ2 and Vβ8.1 genes are said to be associated with Kawasaki's disease, because those T-cells are elevated in patients having that disease. Similarly, T-cells bearing the products of Vβ5.2 and Vβ6.1 genes are associated with MS. The analysis of Tcr variable regions associated with immune system disorders is progressing at a rapid pace.

Upon identification of Tcr variable region genes associated with a particular disease, proteins containing those sequences can be produced by cDNA cloning procedures using published sequence information. Antibody preparations enriched for antibodies which bind to a desired Tcr peptide or conformational determinant may then be prepared by the immunoaffinity procedures described herein.

In one aspect, the method of the present invention utilizes a recombinant single chain Tcr protein (scTcr) for the immunoaffinity purification of antibodies from IVIG and from Cohn Fractions I and III and from Cohn Fraction II. Construction of the scTcr was based upon complete Vα and Vβ regions of the Jurkat T-cell line. The Jurkat cell line is a human monoclonal $CD4^+$, helper α/β^+ leukemia T-cell line, which is available from the American Type Culture Collection, Rockville, Maryland, USA, under Accession No. ATCC 152-T1B. This scTcr molecule contains the Vα and Vβ gene products joined by a linker peptide. The construction of the scTcr from the Jurkat T-cell line using cDNA cloning techniques is described by Lake et al., 1994, supra, incorporated herein by reference. The Lake et al. publication

describes the expression of the scTcr peptide from *E. coli* strain BL21(DE3) transformed with plasmid PET21d.

Recombinant human Tcr proteins may also be used to prepare animal antisera enriched for antibodies which bind to human Tcr or conformational determinants. The animal antisera may be prepared by immunizing animals with Tcr peptides, recombinant human Tcr proteins or human T-cells. The animal antibody preparations so produced have utility as diagnostic reagents.

The novel purified antibodies of this invention may be of any isotype, and those purified from Cohn Fractions I and III are primarily of either IgG or IgM isotype.

The antibody preparations of this invention may be used for diagnosing or monitoring the progress of immune system disorders or conditions, including autoimmune diseases and GVHD. The novel antibody preparations are used diagnostically by determining the extent to which they bind to T-lymphocytes obtained from human subjects. Various immunochemical detection techniques may be used for detecting the interaction of the antibodies and T-lymphocytes. For example, ELISA and flow cytometry using a fluorescent activated cell sorter ("FACS"), as well as other conventional immunochemical procedures, may be used for the detection of the antibody-T-cell interactions.

In addition to their utility for the diagnosis of immune system disorders and conditions, the antibody preparations of this invention have potential therapeutic value. As indicated above, it is known that commercially available immune serum globulin preparations can be used for treating autoimmune diseases and GVHD. In accordance with this invention, it has been shown that these immunoglobulin

preparations contain antibodies to Tcr peptide sequences and conformational determinants. The antibody preparations of this invention are enriched for antibodies to Tcr peptide and conformational determinants up to about 1,000 times over the levels in unprocessed IVIG. Dosages of IVIG used in the treatment of GVHD and autoimmune diseases range from 100 mg to 5 g of IVIG/kg body weight. Based upon *in vitro* and *in vivo* studies (mouse model), effective therapeutic doses of affinity purified antibodies are within the range of about 0.1 mg to about 100 mg/kg of body weight. Such doses can be administered by any suitable methods, with intravenous administration being preferred. These antibody preparations are expected to have numerous advantages over currently-available IVIG preparations in the treatment of these diseases. These advantages arise from the higher potencies and greater selectivities of the antibody preparations of the present invention. Therefore, lower dosages and thus, lower protein loads on the patient can be realized.

This invention is further illustrated by the following examples, which are not intended to be limiting.

EXAMPLE I

Purification of Antibodies from Cohn Fraction I and III

Cohn Fraction I and III was obtained from the Hyland Division of Baxter Healthcare International, Duarte, California, U.S.A. The plasma fraction was centrifuged and dialyzed and then filtered through 0.45 μm filter to remove insoluble Celite and particulates. The resulting solution was subjected to immunoaffinity purification essentially as described by Marchalonis et

al., 1992 supra. The immunoaffinity column was prepared as follows:

BL21 (DE3) *E. coli* cells were purchased from Novagen. PET21d plasmid which contained recombinant scTcr gene was used to transform the BL21 (DE3) *E. coli* so that the *E. coli* would produce the recombinant scTcr protein. The recombinant protein (1.1 milligrams) was dissolved in 10 ml of 0.1 M sodium carbonate (pH 8.0) and incubated with 0.75 g of activated CH Sepharose-4B (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.) at room temperature for 2 hours. The Sepharose was then washed with 40 ml of phosphate-buffered saline (50 mM NaCl/150 mM sodium phosphate, pH 8.0), treated with 40 ml of 1M ethanolamine (pH 8.0) for 1 hour to block unreacted sites, washed with 100 ml of TBS and packed into a 10 cm x 1 cm column.

To remove nonspecific (sticky) antibodies, all samples were first applied to a column packed with ovalbumin immobilized on CH-Sepharose at a flow rate of ~1.2 ml/min. The ovalbumin column previously had been equilibrated with TBS. The effluent from the ovalbumin column was applied directly to the immunoaffinity column (also previously equilibrated with TBS). After washing with 10 bed volumes of TBS, the bound antibodies were eluted with 150 mM glycine-HCl, pH 2.0, collected in 2 ml fractions and immediately neutralized with 3M Tris-NaOH at pH 9.0.

EXAMPLE II

Flow Cytometry

Jurkat cells obtained from ATCC were used to demonstrate cell surface binding of anti-Tcr antibodies. Cells were cultured in RPMI 1640 medium supplemented with 5% fetal calf serum at 37°C in a 95%

air - 5% CO_2 atmosphere. Cells were harvested during exponential growth and separated from the culture supernatant by centrifugation. Cells (1 x 10^6) were resuspended in phosphate buffered saline ("PBS") and combined with 0.5 ml of 10 μg/ml of affinity purified antibodies from an IVIG preparation designated Gammagard® and available from Hyland Division of Baxter Healthcare International, which was purified by the procedure of Example I. An equal number of cells were treated with 0.5 ml of 1:2000 dilution of serum from a rabbit immunized with scTcr (positive control). Cells treated only with buffers (no antibodies) were used as negative controls.

Primary antibody was detected by incubation on ice for one hour with a 1:5000 dilution of goat $(Fab')_2$ anti-human IgG (2° antibody) coupled to fluorescein isothiocyanate ("FITC"). Cells were washed free of 2° antibody with PBS and analyzed on a Becton Dickinson FACscan flow cytometer.

The results are shown in Figures 1-4. Figure 1 represents the instrument output for the negative control, and shows a reactivity of 4.39% positive. Figure 2 represents the positive control and shows a reactivity of 95.44% positive. Figures 3 and 4 represent unpurified IVIG and immunoaffinity purified IVIG respectively. The unpurified antibody mixture had a reactivity of 3.43% positive and the purified antibody preparation had a reactivity of 91.47% positive.

EXAMPLE III

The reactivities of affinity purified antibodies in Cohn Fractions I and III with various human Tcr peptides and with the recombinant Jurkat scTcr

described above were analyzed by ELISA as follows: Hexadecapeptide antigens β3, β8, and β17 corresponded to the first complementarity - determining region, the third framework region and the constant region of the YT35 (Jurkat T cell myeloma cell line) β-chain respectively. Peptide antigens or the scTcr were dissolved in 0.2 M carbonate buffer, pH 9.6. These solutions (100 μg/ml) were added to wells of a microtiter plate and dried overnight at 37°C. Wells were blocked with phosphate-buffered saline, pH 7.4, containing 0.005% Tween 20 (PBST) and 1% gelatin (w/v). Cohn Fraction I + III was reacted with the antigen for one hour followed by washing 4 times with PBST. Peroxidase - conjugated rabbit antibody to human IgM or IgG was used as a developing reagent at a dilution of 1:1000 for the anti-IgM and 2:4000 for the anti-IgG. Conjugate incubations were also for 1 hour. After five washes with PBST, 0.03% substrate (2,2-azino-bis-(3-ethylbenzthioazoline-6-sulfonic acid) in 0.1 M citrate buffer, pH 4.0 and 0.01% hydrogen peroxide v/v were added. Color development was read at 415 nm in a Titertek Multiscan (Flow Labs) after 45 minutes incubation at room temperature. The results are shown in Figure 5. Immunoreactivity was greatest for the β3 peptide followed by scTcr, β8 and β17 in that order. The β3 and β8 peptides were contained in the scTcr, while β17 was not.

EXAMPLE IV

The reactivities of antibodies in sera from four patients with rheumatoid arthritis ("RA") and eight patients with systemic lupus erythematosis ("SLE") with scTcr were analyzed by ELISA using essentially the procedures described in Example III. The results are

shown in Figures 6 and 7 respectively. Significant reactivities with the recombinant scTcr were observed in these patient's sera.

EXAMPLE V

Antibodies which bind to a human Tcr protein, peptide or conformational determinant were affinity purified as in Example I but from a commercial intravenous immunoglobulin (IVIG, Gammagard®) using column chromatography in which peptide β3, corresponding to the first complementarity determining region of a human T cell line (also obtained from YT35?) β chain, was immobilized on CH-Sepharose. Following elution, the antibodies were immediately neutralized with NaOH-glycine. The antibodies then were used in inhibition of phytohaemagglutinin (PHA) stimulated T cell proliferation. The T cell proliferation assay is a standard assay and was performed essentially as in *Current Protocols in Immunology* (Coligan, J.E. et al.; 19940 Series Ed. Richard Coico) vol. I, Section 7.10 (John Wiley and Sons, Inc.) as follows. Serial dilutions of antibodies were pre-incubated at 37°C with normal peripheral blood lymphocytes (PBL) at a concentration of 10^5 cells/well in a 96 well microtiter plate for 30 minutes. 5 μg of PHA were added to the PBL-antibody mixture and the cells were cultured for 72 hours at 37°C. Sixteen hours prior to harvest, one microcurie of tritiated thymidine was added to each well. At the end of the 72 hour incubation, the nuclei were harvested on a cell harvester and counted in a liquid scintillation counter. Percent inhibition by the anti-Tcr antibodies was calculated by the following formula:

1-[(sample-background)/(max. proliferation-background)] X 100%.

The results are shown in Figure 8. Although unprocessed IVIG showed only non-specific background inhibition up to 2500 μg/ml under defined experimental conditions, the affinity purified antibody-induced inhibition increased steadily to 87% as the antibody concentration increased from 3.1 to 25.0 μg/ml. These data demonstrate that antibodies with T cell activity can be specifically purified from commercial IVIG.

EXAMPLE VI

The possible effects of affinity purified antibodies in modulation of T cell activity were studied via interaction with the Tcr in a "proof of principle" model in C57/BL mice. An overview of the experiment is shown in Figure 9. A well-developed sponge model of concomitant tumor immunity was used. The concepts of concomitant tumor immunity are reviewed in Gorelik, E, *Adv. Cancer Res.* 39:71 (1983), and the complete details of the gelatin sponge model used are described in Akporiaye, E.T. et al., *Cancer Res.* 58:1153 (1988), and in Akporiaye, E.T. and K. Muthulakshmi, *Cancer Immunol. Immunother.* 29:199 (1989). In this model, an animal harboring a primary EMT6 mammary tumor is challenged with a secondary tumor implant through a pre-implanted gelatin sponge. During the manifestation of concomitant tumor immunity, the second tumor is rejected and the effector cells can be recovered from the sponge and their tumoricidal activity studied *in vitro*. These cytotoxic tumor rejecting T cells (TRLs) predominantly express Vβ1 and Vβ8 Tcrs. The aim of this experiment was to test the effect of anti-Vβ1 and anti-Vβ8 antibodies affinity

purified from IVIG to modulate *in vitro* the tumoricidal activity of the isolated TRLs.

C57/BL mice were injected on day 0 with Balb/c mouse mammary derived EMT6 tumor cells. On day 8, the mice were surgically implanted with a gelatin sponge and the incisions were allowed to heal. On day 10, fresh EMT6 cells were injected into the gelatin sponges. On day 17, the sponges were removed, digested with gelatinase and the TRLs, which had infiltrated in order to reject the tumor cells, were recovered.

Antibodies which bind to a human Tcr protein, peptide or conformational determinant were affinity purified from IVIG (Gammagard®) using column chromatography with peptides immobilized on CH-Sepharose. The peptides used corresponded to the CDR1 region of Balb/c mouse Vβ1 (EQHLGHNAMY) and Vβ8 (NQTNNHNNMY) Tcr chains. The purification procedure was as described in Example 1. Final preparations of affinity purified antibody were extensively dialyzed against PBS.

Affinity-purified antibodies prepared using each peptide in separate purification steps plus the column flow through IVIG were evaluated in an *in vitro* chromium release assay. The chromium release assay was performed essentially as described in Akporiaye, E.T. and K. Muthulakshni, *Cancer Immunol. Immunother.* 29:199 (1989) with the following modifications. Fifty μl of the TRLs obtained above (1.5×10^5 cells per well) were added to appropriate wells of a 96 well culture plate. Next, 50 μl aliquots (30 μg of antibody per well) were added and the plates incubated for eight hours at 37°C. The degree of cell lysis was determined by measuring the amount of chromium released in the supernatant using a gamma counter.

Figure 10 shows that anti-Vβ1 and anti-Vβ8 independently gave 80% and 50% inhibition of T cell activity, respectively. Added together, they yielded 90% + inhibition. Column flow through as a control showed no inhibition. These data demonstrate that antibodies with specific reactivities are able to inhibit the lytic activity of cytotoxic T-cells via reactivity and modulation of the T-cell receptor. In addition, these data, as a proof of principle, clearly demonstrate the validity of the hypothesis for the ultimate clinical efficacy in the treatment of certain autoimmune disorders.

WHAT IS CLAIMED IS:

1. A human antibody preparation which is enriched for antibodies which bind to a recombinant human Tcr protein.

2. The human antibody preparation of claim 1 which is enriched for antibodies which bind to a recombinant human Tcr protein encoded by a Vα gene or antibodies which bind to a recombinant human Tcr protein encoded by a Vβ gene.

3. The human antibody preparation of claim 1 which is enriched for antibodies which bind to a recombinant human Tcr protein encoded by a Vδ gene or antibodies which bind to a recombinant human Tcr protein encoded by a Vγ gene.

4. The human antibody preparation of claim 1 which is enriched for antibodies to a Tcr peptide or conformational determinant which is associated with an autoimmune disease or condition.

5. The human antibody preparation of claim 4, wherein the autoimmune disease is multiple sclerosis, systemic lupus erythrematosis, Kawasaki [illegible] or rheumatoid arthritis.

6. The antibody preparation of claim [illegible] which is enriched for antibodies which bind to a Tcr peptide or conformational determinant which is associated with graft versus host disease (GVHD).

7. The antibody preparation of claim 1, which is enriched for antibodies which bind to Vα and Vβ peptide sequences of the Jurkat T-cell line, ATCC No. 152-T1B.

8. The antibody preparation of claim 1, which is enriched for antibodies which bind to a single-chain Tcr protein derived from the Vα and Vβ gene sequences of a human T cell.

9. A method for making an antibody preparation which is enriched for antibodies that bind to a human Tcr peptide sequence, which comprises

(a) combining, under antibody-antigen binding conditions, (i) a human plasma Cohn fraction which contains antibodies that bind to a human Tcr peptide or conformational determinant, and (ii) a solid support to which Tcr peptide has been immobilized;

(b) separating unbound proteins from the solid support;

(c) eluting bound antibodies from the solid support under conditions which break the antibody-antigen bonds, thereby forming an antibody preparation which is enriched for antibodies that bind to a human Tcr peptide sequence or conformational determinant.

10. The method of claim 9, wherein the human plasma Cohn fraction employed in step (a) is Cohn Fraction I or III.

11. The method of claim 9, wherein the human plasma Cohn fraction employed in step (a) is Cohn Fractions I and III.

12. The method of claim 9, wherein the human plasma Cohn fraction employed in step (a) is Cohn Fraction II.

13. A method for making an antibody preparation which is enriched for antibodies that bind to a human Tcr peptide or conformational determinant, which comprises:

(a) combining, under antibody-antigen binding conditions, (i) an antibody

mixture which contains antibodies that bind to a human Tcr peptide or conformational determinant and (ii) a solid support to which a recombinant human Tcr protein has been immobilized;

(b) separating unbound proteins from the solid support;

(c) eluting bound antibodies from the solid support under conditions which break the antibody-antigen bonds, thereby forming an antibody preparation which is enriched for antibodies that bind to a human Tcr peptide or conformational determinant.

14. The method of claim 13, wherein the antibody mixture employed in step (a) is a human antibody mixture.

15. The method of claim 13, wherein the antibody mixture employed in step (a) is an animal antibody mixture.

16. The method of claim 14, wherein the antibody mixture is a human plasma Cohn fraction.

17. The method of claim 16, wherein the antibody mixture is Cohn Fraction I and III.

18. The method of claim 16, wherein the antibody mixture is Cohn Fraction II.

19. The method of claim 14, wherein the antibody mixture employed in step (a) is a human IVIG preparation.

20. The method of claim 13, wherein the immobilized recombinant human Tcr protein employed in step (a) is a protein encoded by a region of a Vα, Vβ, Vδ or Vγ gene.

21. The method of claim 20, wherein the recombinant human Tcr protein is a single-chain Tcr protein derived from the Vα and Vβ gene sequences of a human T cell.

22. A method of diagnosing a human for an autoimmune disease or condition or GVHD which comprises

(a) identifying a Tcr peptide or conformational determinant that is elevated in humans having said autoimmune disease or condition or GVHD;

(b) combining T-lymphocytes from said human with a human antibody preparation which is enriched for antibodies which bind to said Tcr peptide or conformational determinant;

(c) determining the extent to which antibodies in said antibody preparation bind to the said T-lymphocytes.

23. The method of claim 22, wherein the autoimmune disease is multiple sclerosis, systemic lupus erythematosis, Kawasaki's disease or rheumatoid arthritis.

24. The method of claim 22, wherein the Tcr protein or conformational determinant is encoded by a region of a Vα or Vδ gene.

25. The method of claim 22, wherein the Tcr peptide or conformational determinant is encoded by a region of a Vβ or γ gene.

26. The method of claim 22, wherein step (c) is accomplished by means of an ELISA procedure.

27. The method of claim 22, wherein step (c) is accomplished by fluorescent flow cytometry.

28. A method of treating a patient suffering from an autoimmune disease or condition or graft versus host

disease (GVHD) which comprises administering to the patient a human antibody preparation which is enriched for antibodies which bind to a Tcr peptide or conformational determinant that is present in elevated levels of circulating T cells in patients having said autoimmune disease or GVHD, wherein said preparation comprises a sufficient amount of said antibodies to bind to said peptide or conformational determinant.

29. The method of claim 28, wherein the antibodies are administered in a dosage range of about 0.1 to about 100 mg/kg body weight.

30. The method of claim 28, wherein the antibody preparation is enriched for antibodies which bind to a recombinant human Tcr protein encoded by a Vα gene or antibodies which bind to a recombinant human Tcr protein encoded by a Vβ gene.

31. The method of claim 28, wherein the antibody preparation is enriched for antibodies which bind to a recombinant human Tcr protein encoded by a Vδ gene or antibodies which bind to a recombinant human Tcr protein encoded by a Vγ gene.

32. The method of claim 28, wherein the disease is an autoimmune disease.

33. The method of claim 30, wherein the autoimmune disease comprises Kawasaki's disease, multiple sclerosis, rheumatoid arthritis or systemic lupus erythematosis.

34. The method of claim 28, wherein the disease is GVHD.

FIG. 1

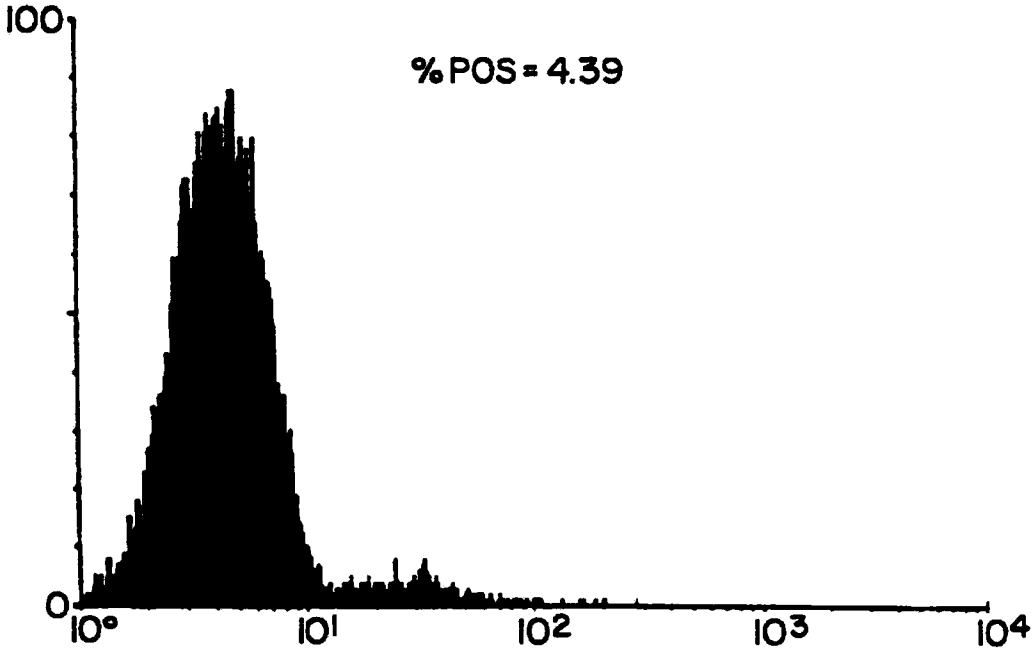

100
%POS = 4.39
0
10°
10¹
10²
10³
10⁴

FIG. 2

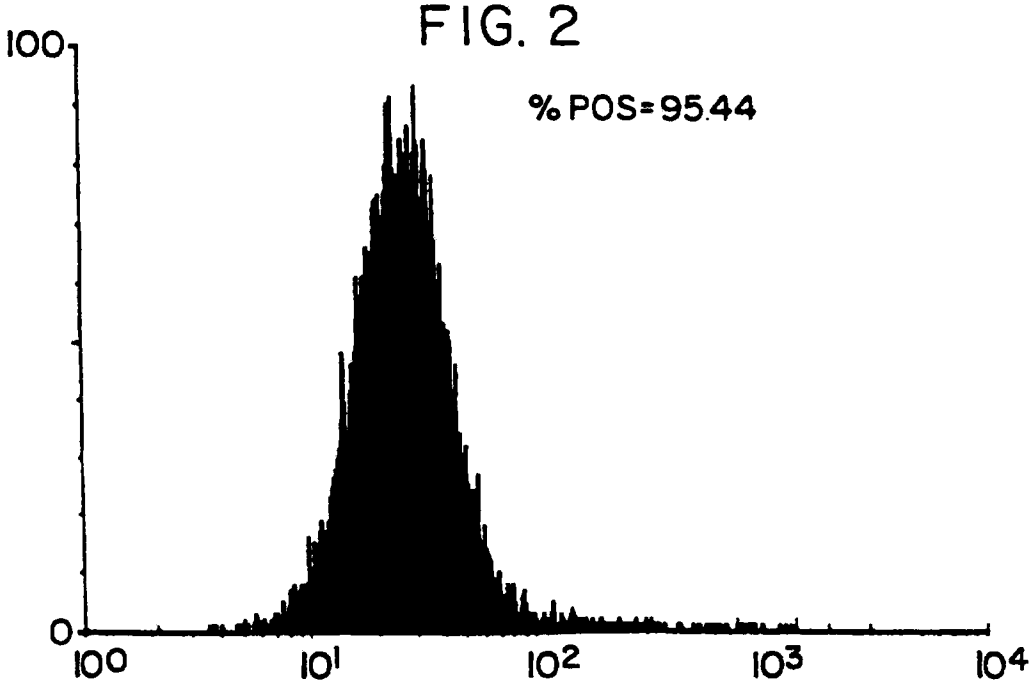

100
%POS = 95.44
0
10⁰
10¹
10²
10³
10⁴

FIG. 3

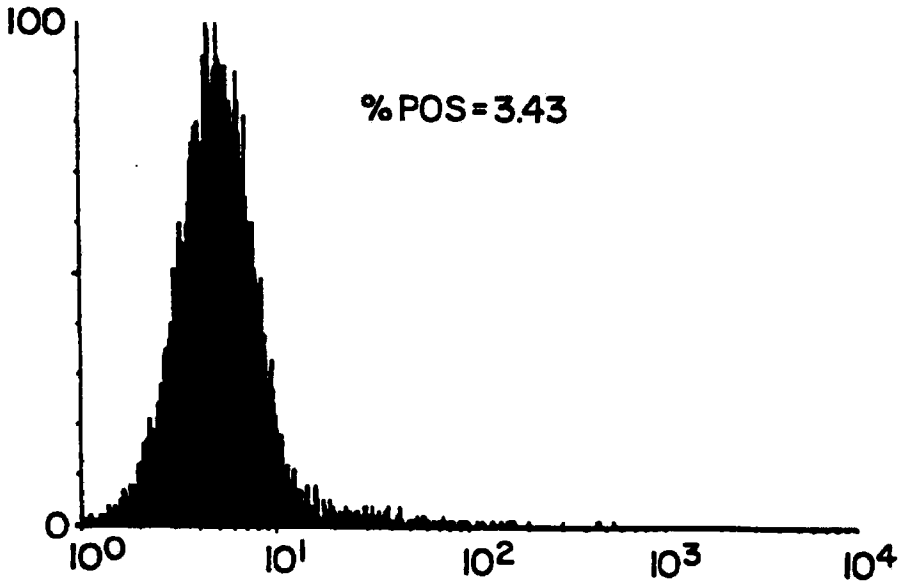

100
%POS=3.43
0
10^0
10^1
10^2
10^3
10^4

FIG. 4

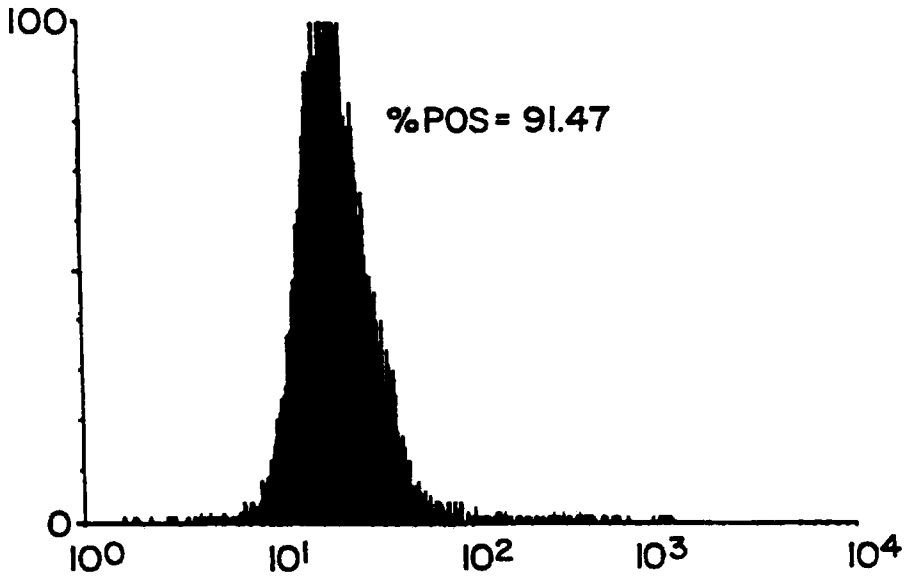

100
%POS= 91.47
0
10^0
10^1
10^2
10^3
10^4

FIG. 5

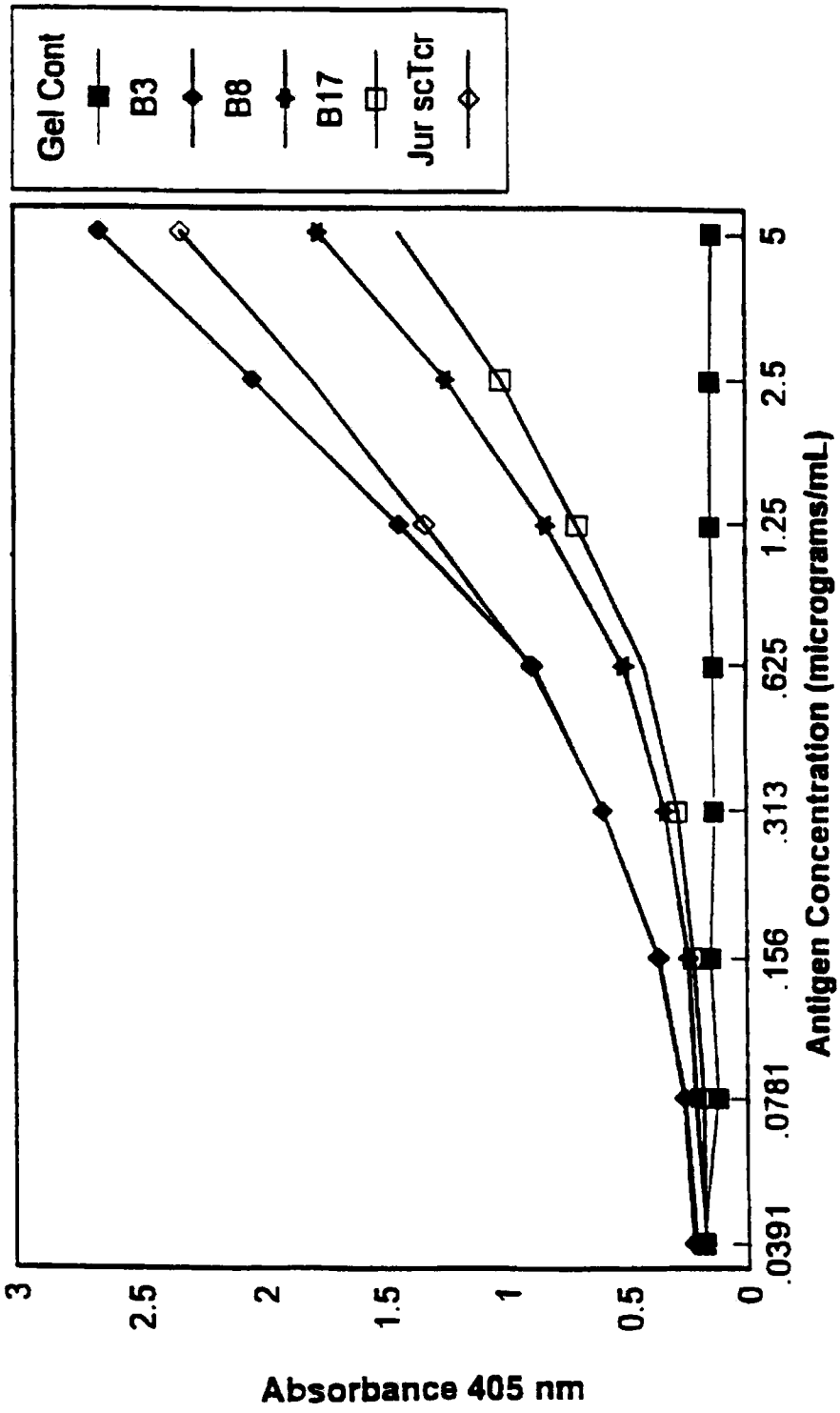

Gel Cont
B3
B8
B17
Jur scTcr
Absorbance 405 nm
0
0.5
1
1.5
2
2.5
3
Antigen Concentration (micrograms/mL)
.0391
.0781
.156
.313
.625
1.25
2.5
5

FIG. 6

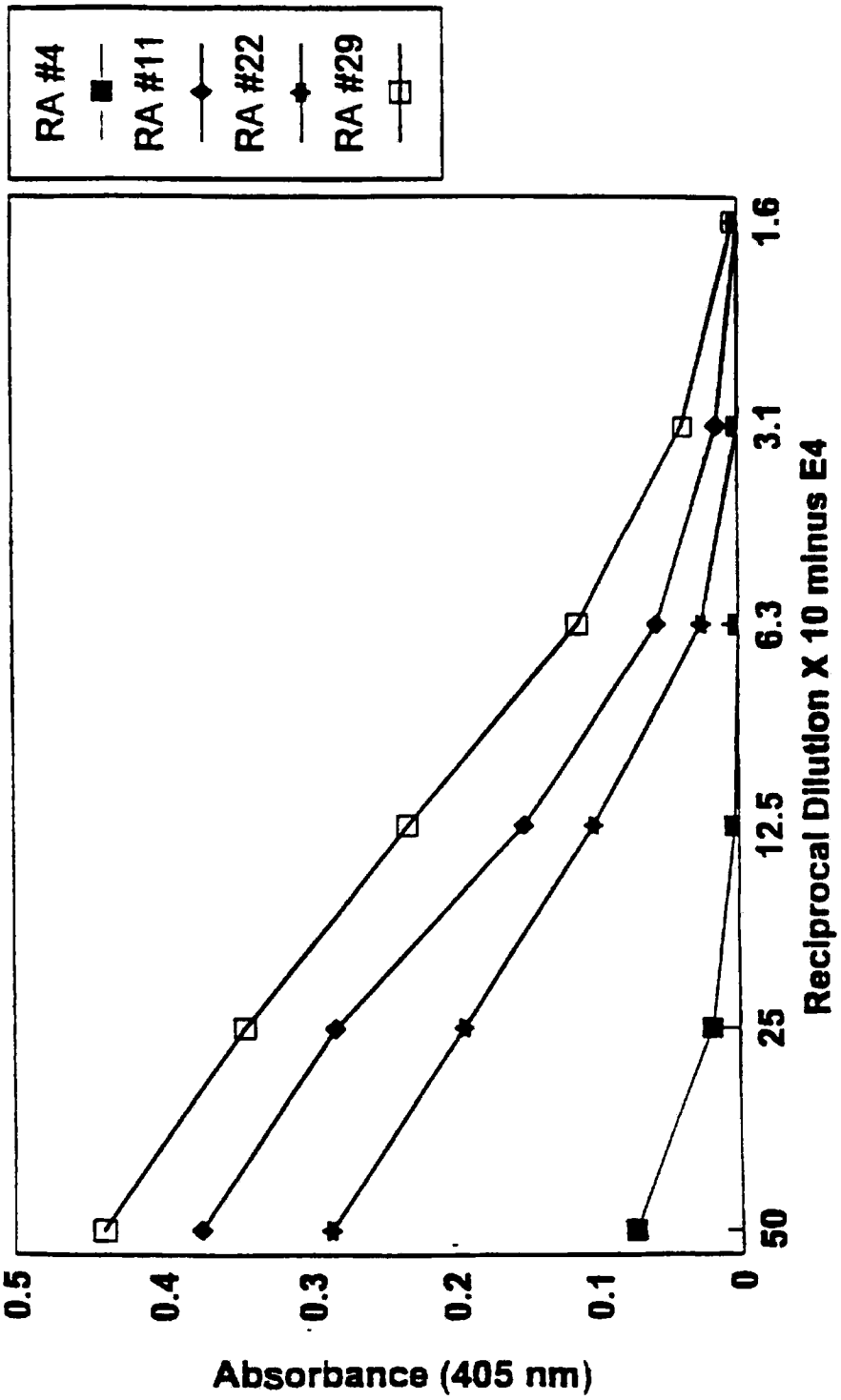

RA #4
RA #11
RA #22
RA #29
Reciprocal Dilution X 10 minus E4
1.6
3.1
6.3
12.5
25
50
0
0.1
0.2
0.3
0.4
0.5
Absorbance (405 nm)

FIG. 7

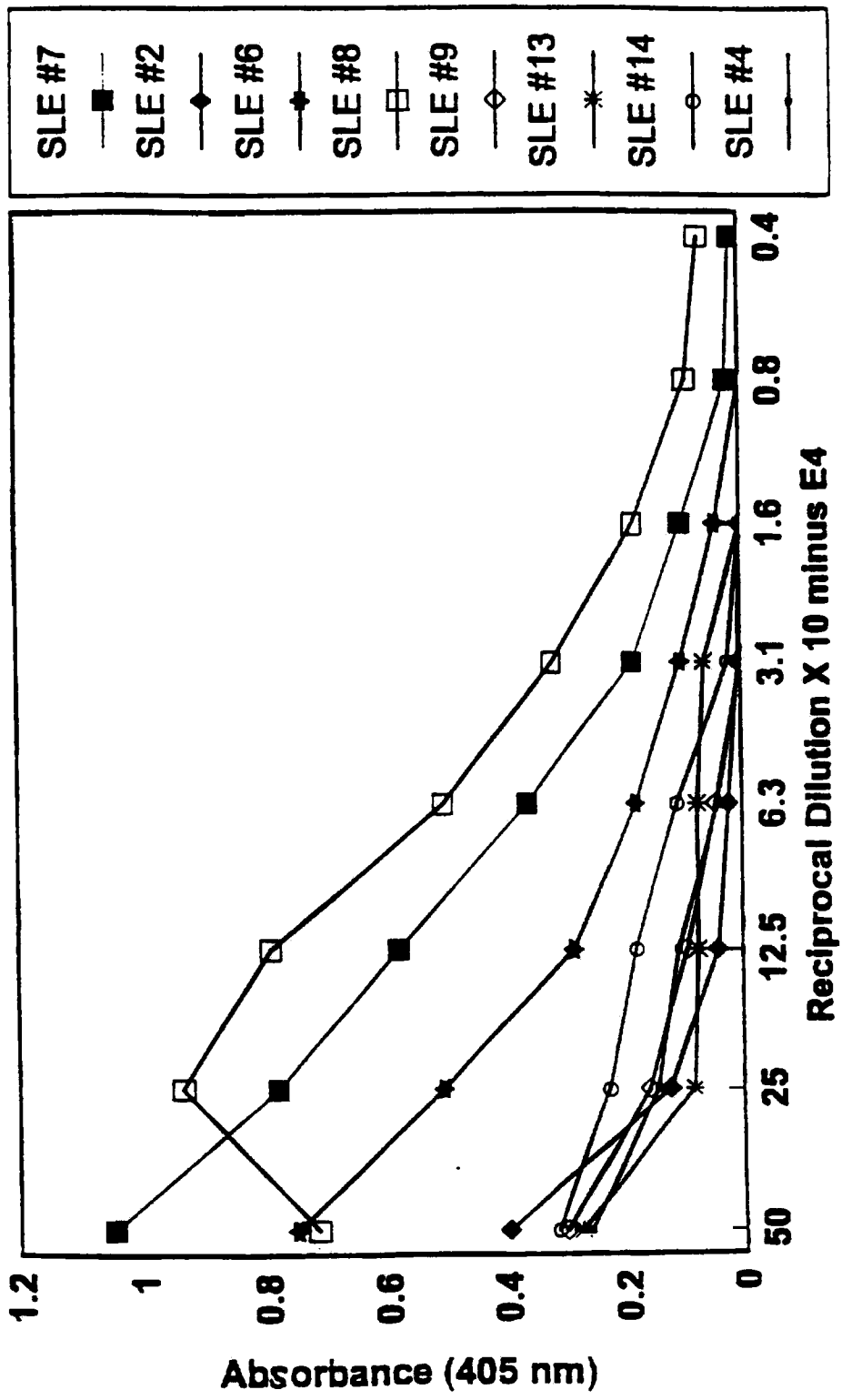

SLE #7
SLE #2
SLE #6
SLE #8
SLE #9
SLE #13
SLE #14
SLE #4
Reciprocal Dilution X 10 minus E4
0.4
0.8
1.6
3.1
6.3
12.5
25
50
0
0.2
0.4
0.6
0.8
1
1.2
Absorbance (405 nm)

FIG. 8

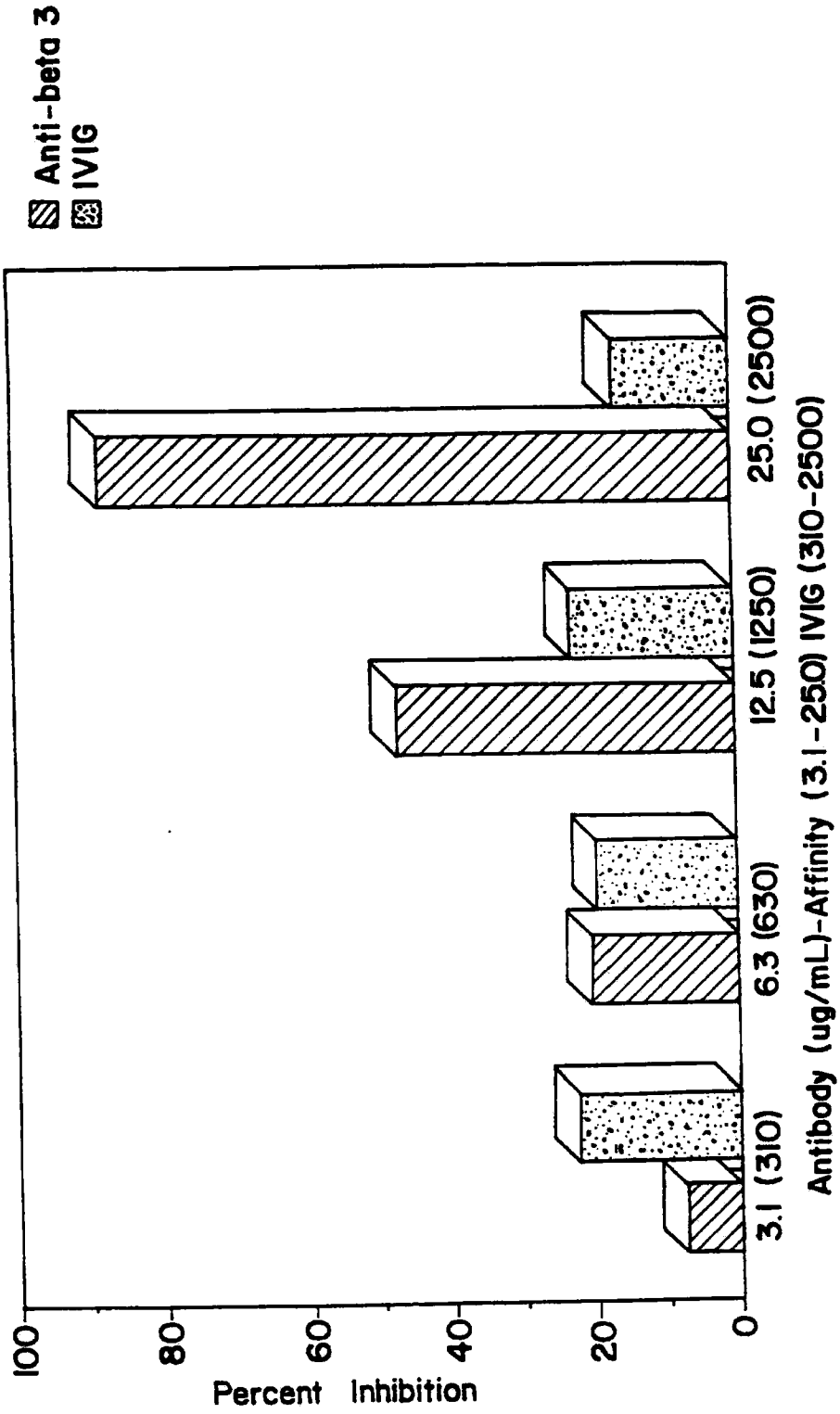

Anti-beta 3
IVIG
100
80
60
40
20
0
Percent Inhibition
3.1 (310)
6.3 (630)
12.5 (1250)
25.0 (2500)
Antibody (ug/mL)-Affinity (3.1-250) IVIG (310-2500)

FIG. 9

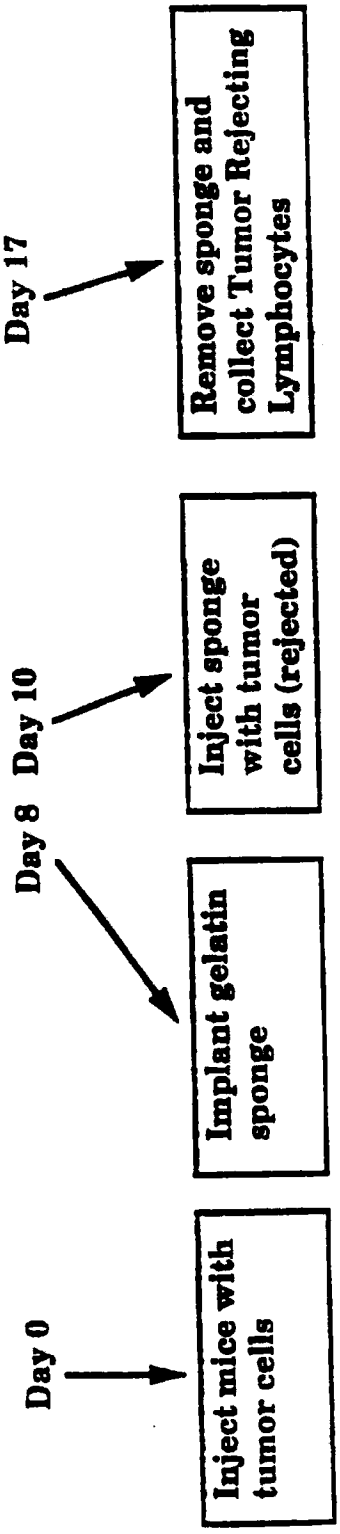
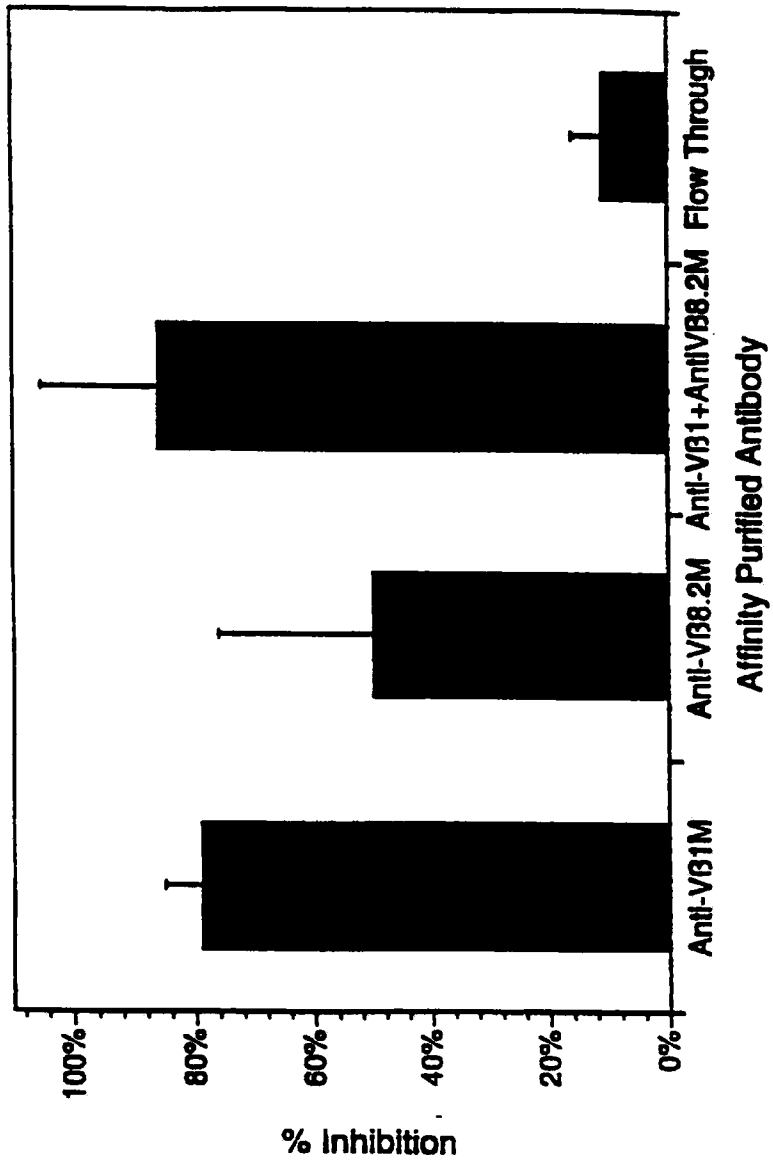

Day 0
Inject mice with tumor cells
Day 8
Implant gelatin sponge
Day 10
Inject sponge with tumor cells (rejected)
Day 17
Remove sponge and collect Tumor Rejecting Lymphocytes

FIG. 10


Anti-Vβ1M
Anti-Vβ8.2M
Anti-Vβ1+AntiVβ8.2M
Flow Through
Affinity Purified Antibody
100%
80%
60%
40%
20%
0%
% Inhibition

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 95/14869

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K16/28 C07K1/22 G01N33/564 G01N33/68 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 89, no. 8, 15 April 1992 WASHINGTON, DC, USA, pages 3325-3329, J. MARCHALONIS ET AL. 'Human autoantibodies reactive with synthetic autoantigens from T-cell receptor beta chain.' cited in the application see abstract see conclusions and speculations --- -/--	1-34

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
29 February 1996	25.03.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CLINICAL PHARMACY, vol. 9, no. 7, July 1990 BETHESDA, MD, USA, pages 509-529, M. KNAPP ET AL. 'Clinical uses of intravenous immune globulin.' see the whole document	1-34
A	PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, vol. 207, no. 2, November 1994 NEW YORK, NY, USA, pages 129-147, J. MARCHALONIS ET AL. 'Synthetic autoantigens of immunoglobulins and T-cell receptors: Their recognition in aging, infection, and autoimmunity.' see concluding remarks	1-34
A	ANNALES DE MÉDECINE INTERNE, vol. 144, no. 8, 1993 PARIS, FRANCE, pages 506-513, L. MOUTHON ET AL. 'Mécanismes d'action des immunoglobulines intraveineuses dans la traitement des pathologies auto-immunes (Mechanisms of action of intravenous immunoglobulins (IgIV) in the treatment of autoimmune diseases).' see abstract see tables see page 510, right column, line 5 - page 511, left column, line 24	1-34
A	SEMINARS IN HEMATOLOGY, vol. 29, no. 3 suppl. 2, July 1992 NEW YORK, NY, USA, pages 64-71, S. KAVERI ET AL. 'Can intravenous immunoglobulin treatment regulate autoimmune responses.' see the whole document	1-34
A	EP,A,0 403 156 (GENZYME CORPORATION) 19 December 1990 see examples see claims	1,2,8
A	WO,A,91 16910 (SYSTEMIX, INC.) 14 November 1991 see page 10, line 14 - line 31	1

1

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 95/14869

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, vol. 383, 1995 NEW YORK, NY, USA, pages 223-229, D. LAKE ET AL. 'Characterization of autoantibodies directed against T cell receptors.' see the whole document	1,2,4,5, 7-11, 13-17, 19-30, 32,33

1

INTERNATIONAL SEARCH REPORT

:rnational application No.
PCT/US95/14869

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 28-34
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: ALTHOUGH CLAIMS 28-34 ARE DIRECTED TO A METHOD OF TREATMENT OF THE HUMAN/ANIMAL BODY, THE SEARCH HAS BEEN CARRIED OUT AND BASED ON THE ALLEGED EFFECTS OF THE COMPOSITION/COMPOUND.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 95/14869

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-403156	19-12-90	CA-A-	2018248	07-12-90
		JP-A-	3219896	27-09-91
WO-A-9116910	14-11-91	AU-B-	637914	10-06-93
		AU-B-	7858391	27-11-91
		CA-A-	2063408	04-11-91
		EP-A-	0481058	22-04-92
		JP-T-	4507109	10-12-92
		US-A-	5411749	02-05-95